(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 452 603 A1

(12) EUROPEAN PATENT APPLICATION
published in accordance with Art. 158(3) EPC

(43) Date of publication:
01.09.2004 Bulletin 2004/36

(21) Application number: 02800753.2

(22) Date of filing: 03.10.2002

(51) Int Cl.7: C12P 13/00

(86) International application number:
PCT/JP2002/010312

(87) International publication number:
WO 2003/031636 (17.04.2003 Gazette 2003/16)

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 05.10.2001 JP 2001309682

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(54) PROCESS FOR PRODUCTION OF OPTICALLY ACTIVE 3-HYDROXY- PENTANENITRILE

(57) The present invention provides a method for preparing optically active 3-hydroxypentanenitrile with high yield. Optically active 3-hydroxypentanenitrile is prepared by stereoselectively reducing 3-ketopentanenitrile by action of an enzyme, which asymmetrically reduces 3-ketopentanenitrile to optically active 3-hydroxypentanenitrile. Also, alkali metal salt of 3-ketopentanenitrile, which is a stable compound without problems regarding storage, can be efficiently obtained.

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Printed by Jouve, 75001 PARIS (FR)

Description

TECHNICAL FIELD

[0001] The present invention relates to a process for preparing optically active 3-hydroxypentanenitrile. Optically active 3-hydroxypentanenitrile is a compound that is useful as a synthetic raw material and an intermediate of pharmaceutical products or agricultural chemicals, which require optical activity.

BACKGROUND ART

[0002] As a process for preparing optically active 3-hydroxypentanenitrile, known is the optical resolution method (J. Org. Chem., 62, 9165 (1997)), wherein 3-acetoxynitrile compound which is a racemic body is hydrolyzed in the presence of thio-crown ether using a lipase derived from Pseudomonas cepacia. However, because this method is an optical resolution method, the yield of one enantiomer is low, that is at most 50 %, and therefore is not satisfactory. Also, because the optical purity of the produced 3-hydroxypentanenitrile is low and thio-crown ether is added to improve the discrimination of an enzyme, industrial operation is difficult when considering cost and safety.

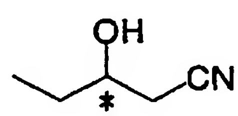
[0003] Also, a method for synthesizing 3-ketopentanenitrile, which is used as a raw material for preparing optically active 3-hydroxypentanenitrile in the present invention, is already known (WO94/21617). However, the obtained 3-ketopentanenitrile is known to be an unstable compound and to polymerize on its own (Aust. J. Chem., 44, 1263, (1991)). Therefore, 3-ketopentanenitrile is difficult to store over a long period of time and difficult to use from an industrial viewpoint.

[0004] As a result of intensive studies to develop an efficient process for preparing optically active 3-hydroxypentanenitrile, the present inventors have newly discovered an enzyme source, which has ability to stereoselectively reduce and convert 3-ketopentanenitrile into optically active 3-hydroxypentanenitrile. Thus, the present invention was achieved.

[0005] Furthermore, as a result of studies focusing on alkali metal salt of 3-ketopentanenitrile in order to avoid problems regarding storage due to unstableness of 3-ketopentanenitrile, a process for efficiently obtaining alkali metal salt of 3-ketopentanenitrile, which is a stable compound without problems regarding storage, has been discovered. Thus, the present invention was achieved.

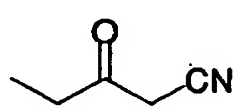
DISCLOSURE OF INVENTION

[0006] That is, the present invention relates to a process for preparing optically active 3-hydroxypentanenitrile represented by the following formula (1):



(1)

wherein an enzyme, which asymmetrically reduces 3-ketopentanenitrile to optically active 3-hydroxypentanenitrile, acts upon 3-ketopentanenitrile represented by the following formula (2):



(2)

to obtain optically active 3-hydroxypentanenitrile.

[0007] The enzyme is preferably an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Arthroascus genus, Candida genus, Cryptococcus genus, Debaryomyces genus, Dekkera genus, Dipodascus genus, Geotrichum genus, Guilliermondella genus, Hyphopichia genus, Issatchenkia genus, Kluyveromyces genus, Komagataella genus, Lipomyces genus, Lodderomyces genus, Met-

schnikowia genus, Ogataea genus, Pichia genus, Rhodotorula genus, Rhodsporidium genus, Schizoblastosporion genus, Schwanniomyces genus, Stephanoascus genus, Torulaspora genus, Trichosporon genus, Williopsis genus, Yarrowia genus, Acidephilium genus, Agrobacterium genus, Alcaligenes genus, Arthrobacter genus, Brevundimonas genus, Cellulomonas genus, Comamonas genus, Microbacterium genus, Paenibacillus genus, Rhodococcus genus, Citeromyces genus, Achromobacter genus, Corynebacterium genus, Devosia genus, Hofnia genus, Proteus genus, Providencia genus, Pseudomonas genus, Absidia genus, Aegerita genus, Agrocybe genus, Amylostereum genus, Aspergillus genus, Corynascucs genus, Dendryphiella genus, Emericella genus, Fusarium genus, Gibberella genus, Glomerella genus, Macrophoma genus, Micronectriella genus, Mortierella genus, Mucor genus, Nannizzia genus, Penicillium genus, Phialophora genus, Rhizopus genus, Sclerotinia genus, Sclerotium genus and Streptomyces genus; and/or a purified enzyme obtained from the microorganism.

[0008] The absolute configuration of the produced optically active 3-hydroxypentanenitrile is preferably R-configuration and the enzyme is preferably an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Arthroascus genus, Candida genus, Cryptococcus genus, Debaryomyces genus, Dekkera genus, Geotrichum genus, Guilliermondella genus, Issatchenkia genus, Kluyveromyces genus, Komagataella genus, Lipomyces genus, Lodderomyces genus, Metschnikowia genus, Ogataea genus, Pichia genus, Rhodotorula genus, Rhodsporidium genus, Schwanniomyces genus, Stephanoascus genus, Torulaspora genus, Trichosporon genus, Williopsis genus, Yarrowia genus, Acidephilium genus, Agrobacterium genus, Alcaligenes genus, Arthrobacter genus, Cellulomonas genus, Comamonas genus, Microbacterium genus, Rhodococcus genus, Citeromyces genus, Achromobacter genus, Corynebacterium genus, Devosia genus, Hofnia genus, Proteus genus, Providencia genus, Absidia genus, Aegerita genus, Agrocybe genus, Amylostereum genus, Aspergillus genus, Corynascucs genus, Dendryphiella genus, Emericella genus, Fusarium genus, Gibberella genus, Glomerella genus, Macrophoma genus, Micronectriella genus, Mortierella genus, Mucor genus, Nannizzia genus, Penicillium genus, Phialophora genus, Rhizopus genus, Sclerotinia genus, Sclerotium genus and Streptomyces genus; and/or a purified enzyme obtained from the microorganism.

[0009] The absolute configuration of the produced optically active 3-hydroxypentanenitrile is preferably R-configuration and the enzyme is preferably an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Arthroascus javanensis, Candida cantarellii, Candida fennica, Candida glabrata, Candida gropengiesseri, Candida kefyr, Candida maris, Candida melinii, Candida musae, Candida pararugosa, Candida pinus, Candida sorbophila, Candida tenuis, Candida utilis, Cryptococcus curvatus, Cryptococcus humicolus, Debaryomyces hansenii, Debaryomyces hansenii var. fabryi, Debaryomyces hansenii var. hansenii, Debaryomyces marama, Debaryomyces nepalensis, Dekkera anomala, Geotrichum candidum, Geotrichum eriense, Geotrichum fermentans, Guilliermondella selenospora, Issatchenkia orientalis, Issatchenkia terricola, Kluyveromyces marxianus, Komagataella pastoris, Lipomyces starkeyi, Lodderomyces elongisporus, Metschnikowia bicuspidata, Metschnikowia gruessii, Ogataea pini, Ogataea wickerhamii, Pichia anomala, Pichia canadensis, Pichia jadinii, Pichia petersonii, Pichia rhodanensis, Pichia silvicola, Pichia triangularis, Rhodotorula lactosa, Rhodotorula rubra, Rhodsporidium diobovatum, Rhodsporidium sphaerocarpum, Rhodsporidium toruloides, Schwanniomyces occidentalis var. occidentalis, Stephanoascus ciferrii, Torulaspora delbrueckii, Trichosporon cutaneum, Williopsis saturnus var. mrakii, Williopsis saturnus var. saturnus, Williopsis saturnus var. suaveolens, Yarrowia lipolytica, Acidephilium cryptum, Agrobacterium tumefacience, Alcaligenes sp., Achromobacter xylosoxidans subsp. denitrificans, Arthrobacter protophormiae, Cellulomonas gelida, Comamonas testosteroni, Microbacterium arborescens, Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus rhodochrous, Candida magnoliae, Citeromyces matritensis, Pichia bispora, Trichosporon loubieri var. loubieri, Corynebacterium ammoniagenes, Corynebacterium flavescens, Devosia riboflavina, Hofnia alvei, Proteus vulgaris, Providencia alcalifaciens, Absidia coerulea, Absidia hyalospora, Aegerita candida, Agrocybe cylyndracea, Amylostereum areolatum, Aspergillus niger, Aspergillus phoenicis, Aspergillus sojae, Corynascucs sepedonium, Dendryphiella salina, Emericella nidulans var. nidulans, Emericella unguis, Fusarium oxysporum, Fusarium anguloides, Gibberella fujikuroi, Glomerella cingulata, Macrophoma commelinae, Micronectriella cucumeris, Mortierella isabellina, Mortierella ramanniana var. angulispora, Mucor tuberculisporus, Mucor inaequisporus, Nannizzia gypsea var. incurvata, Penicillium chermesium, Penicillium expansum, Phialophora fastigiata, Rhizopus niveus, Rhizopus oryzae, Sclerotinia sclerotiorum, Sclerotium delphinii, Streptomyces cacaoi subsp. asoensis and Streptomyces sp.; and/or a purified enzyme obtained from the microorganism.

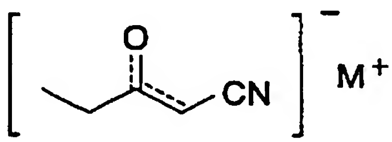
[0010] The absolute configuration of the produced optically active 3-hydroxypentanenitrile is preferably S-configuration and the enzyme is preferably an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Candida genus, Dipodascus genus, Geotrichum genus, Hyphopichia genus, Kluyveromyces genus, Pichia genus, Schizoblastosporion genus, Schwanniomyces genus, Brevundimonas genus, Paenibacillus genus, Rhodotorula genus, Pseudomonas genus and Streptomyces genus; and/or a purified enzyme obtained from the microorganism.

[0011] The absolute configuration of the produced optically active 3-hydroxypentanenitrile is preferably S-configuration and the enzyme is preferably an enzyme present in a cell, a culture solution or a treated substance thereof of a

microorganism selected from the group consisting of Candida albicans, Candida haemulonii, Candida intermedia, Candida maltosa, Candida mogii, Candida oleophila, Dipodascus ovetensis, Dipodascus tetrasperma, Geotrichum fragrans, Hypopichia burtonii, Kluyveromyces polysporus, Pichia stipitis, Schizoblastosporion kobayasii, Schwanniomyces occidentalis var. occidentalis, Brevundimonas diminuta, Paenibacillus alvei, Rhodotorula glutinis var. dairenensis, Pseudomonas stutzeri, Pseudomonas mendocina, Streptomyces coelescens and Streptomyces hydrogenans; and/or a purified enzyme obtained from the microorganism.

[0012] Either or both of oxidized nicotinamide adenine dinucleotide (NAD^+) and oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) preferably coexists with an enzyme that reduces each to a reduced form and a substrate for reducing.

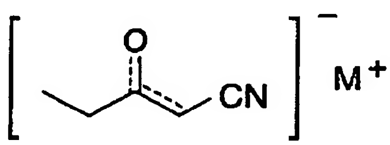
[0013] An alkali metal salt of 3-ketopentanenitrile represented by the following formula (3):



(3)

(wherein M represents an alkali metal) is preferably used as 3-ketopentanenitrile.

[0014] The present invention also relates to a process for preparing an alkali metal salt of 3-ketopentanenitrile, which comprises synthesizing 3-ketopentanenitrile from propionic acid ester and acetonitrile in the presence of an alkali metal base; and obtaining 3-ketopentanenitrile from the reaction system as an alkali metal salt represented by the following formula (3):



(3)

(wherein M represents an alkali metal).

BEST MODE FOR CARRYING OUT THE INVENTION

[0015] The present invention is described below.

[0016] The 3-ketopentanenitrile used as the substrate of the present invention can be synthesized by the method described in WO94/21617.

[0017] The enzyme used in the present invention is an enzyme that converts 3-ketopentanenitrile into optically active 3-hydroxypentanenitrile. Specifically, examples are reductase and dehydrogenase, which reduce a carbonyl group into a hydroxyl group. These enzymes are present in a cell, a culture solution or a treated substance of the cell or are purified and in the present invention, these may be used alone or in a combination of two or more kinds.

[0018] A microorganism having ability to convert 3-ketopentanenitrile into optically active 3-hydroxypentanenitrile can be found, for example, from the method described below. A test tube is charged with 5 ml of a liquid medium (pH 7) comprising 40 g of glucose, 3 g of yeast extract, 6.5 g of diammonium hydrogenphosphate, 1 g of potassium dihydrogenphosphate, 0.8 g of magnesium sulfate heptahydrate, 60 mg of zinc sulfate heptahydrate, 90 mg of iron sulfate heptahydrate, 5 mg of copper sulfate pentahydrate, 10 mg of manganese sulfate tetrahydrate and 100 mg of sodium chloride (all per 1 L) and sterilized. Then, the microorganism is aseptically inoculated and cultured by shaking at 30°C for 2 to 3 days. Subsequently, the cells are collected by centrifugation and suspended in 1 to 5 ml of a phosphate buffer solution containing 2 to 10 % of glucose. The suspension is added to a test tube in which 2.5 to 25 mg of 3-ketopentanenitrile is added in advance and shaken for 2 to 3 days at 30°C. At this time, a substance obtained by drying the centrifugalized cells in a desiccator or by acetone can also be used.

[0019] The microorganism used in the present invention can be any microorganism, as long as the microorganism has ability to convert 3-ketopentanenitrile into optically active 3-hydroxypentanenitrile. Examples are microorganisms belonging to Arthroascus genus, Candida genus, Cryptococcus genus, Debaryomyces genus, Dekkera genus, Dipodascus genus, Geotrichum genus, Guilliermondella genus, Hyphopichia genus, Issatchenkia genus, Kluyveromyces

genus, Komagataella genus, Lipomyces genus, Lodderomyces genus, Metschnikowia genus, Ogataea genus, Pichia genus, Rhodotorula genus, Rhodsporidium genus, Schizoblastosporion genus, Schwanniomyces genus, Stephanoascus genus, Torulaspora genus, Trichosporon genus, Williopsis genus, Yarrowia genus, Acidephilium genus, Agrobacterium genus, Alcaligenes genus, Arthrobacter genus, Brevundimonas genus, Cellulomonas genus, Comamonas genus, Microbacterium genus, Paenibacillus genus, Rhodococcus genus, Citeromyces genus, Achromobacter genus, Corynebacterium genus, Devosia genus, Hofnia genus, Proteus genus, Providencia genus, Pseudomonas genus, Absidia genus, Aegerita genus, Agrocybe genus, Amylostereum genus, Aspergillus genus, Corynascucs genus, Dendryphiella genus, Emericella genus, Fusarium genus, Gibberella genus, Glomerella genus, Macrophoma genus, Micronectriella genus, Mortierella genus, Mucor genus, Nannizzia genus, Penicillium genus, Phialophora genus, Rhizopus genus, Sclerotinia genus, Sclerotium genus and Streptomyces genus.

[0020] Particularly, when converting into 3-hydroxypentanenitrile whose absolute configuration is R-configuration, preferable microorganisms are microorganisms belonging to Arthroascus genus, Candida genus, Cryptococcus genus, Debaryomyces genus, Dekkera genus, Geotrichum genus, Guilliermondella genus, Issatchenkia genus, Kluyveromyces genus, Komagataella genus, Lipomyces genus, Lodderomyces genus, Metschnikowia genus, Ogataea genus, Pichia genus, Rhodotorula genus, Rhodsporidium genus, Schwanniomyces genus, Stephanoascus genus, Torulaspora genus, Trichosporon genus, Williopsis genus, Yarrowia genus, Acidephilium genus, Agrobacterium genus, Alcaligenes genus, Arthrobacter genus, Cellulomonas genus, Comamonas genus, Microbacterium genus, Rhodococcus genus, Citeromyces genus, Achromobacter genus, Corynebacterium genus, Devosia genus, Hofnia genus, Proteus genus, Providencia genus, Absidia genus, Aegerita genus, Agrocybe genus, Amylostereum genus, Aspergillus genus, Corynascucs genus, Dendryphiella genus, Emericella genus, Fusarium genus, Gibberella genus, Glomerella genus, Macrophoma genus, Micronectriella genus, Mortierella genus, Mucor genus, Nannizzia genus, Penicillium genus, Phialophora genus, Rhizopus genus, Sclerotinia genus, Sclerotium genus and Streptomyces genus. Further preferable examples are Arthroascus javanensis, Candida cantarellii, Candida fennica, Candida glabrata, Candida gropengiesseri, Candida kefyr, Candida maris, Candida melinii, Candida musae, Candida pararugosa, Candida pinus, Candida sorbophila, Candida tenuis, Candida utilis, Cryptococcus curvatus, Cryptococcus humicolus, Debaryomyces hansenii, Debaryomyces hansenii var. fabryi, Debaryomyces hansenii var. hansenii, Debaryomyces marama, Debaryomyces nepalensis, Dekkera anomala, Geotrichum candidum, Geotrichum eriense, Geotrichum fermentans, Guilliermondella selenospora, Issatchenkia orientalis, Issatchenkia terricola, Kluyveromyces marxianus, Komagataella pastoris, Lipomyces starkeyi, Lodderomyces elongisporus, Metschnikowia bicuspidata, Metschnikowia gruessii, Ogataea pini, Ogataea wickerhamii, Pichia anomala, Pichia canadensis, Pichia jadinii, Pichia petersonii, Pichia rhodanensis, Pichia silvicola, Pichia triangularis, Rhodotorula lactosa, Rhodotorula rubra, Rhodsporidium dioboyatum, Rhodsporidium sphaerocarpum, Rhodsporidium toruloides, Schwanniomyces occidentalis var. occidentalis, Stephanoascus ciferrii, Torulaspora delbrueckii, Trichosporon cutaneum, Williopsis saturnus var. mrakii, Williopsis saturnus var. saturnus, Williopsis saturnus var. suaveolens, Yarrowia lipolytica, Acidephilium cryptum, Agrobacterium tumefacience, Alcaligenes sp., Achromobacter xylosoxidans subsp. denitrificans, Arthrobacter protophormiae, Cellulomonas gelida, Comamonas testosteroni, Microbacterium arborescens, Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus rhodochrous, Candida magnoliae, Citeromyces matritensis, Pichia bispora, Trichosporon loubieri var. loubieri, Corynebacterium ammoniagenes, Corynebacterium flavescens, Devosia riboflavina, Hofnia alvei, Proteus vulgaris, Providencia alcalifaciens, Absidia coerulea, Absidia hyalospora, Aegerita candida, Agrocybe cylyndracea, Amylostereum areolatum, Aspergillus niger, Aspergillus phoenicis, Aspergillus sojae, Corynascucs sepedonium, Dendryphiella salina, Emericella nidulans var. nidulans, Emericella unguis, Fusarium oxysporum, Fusarium anguioides, Gibberella fujikuroi, Glomerella cingulata, Macrophoma commelinae, Micronectriella cucumeris, Mortierella isabellina, Mortierella ramanniana var. angulispora, Mucor tuberculisporus, Mucor inaequisporus, Nannizzia gypsea var. incurvata, Penicillium chermesium, Penicillium expansum, Phialophora fastigiata, Rhizopus niveus, Rhizopus oryzae, Sclerotinia sclerotiorum, Sclerotium delphinii, Streptomyces cacaoi subsp. asoensis and Streptomyces sp.

[0021] When converting into 3-hydroxypentanenitrile whose absolute configuration is S-configuration, preferable microorganisms are microorganisms belonging to Candida genus, Dipodascus genus, Geotrichum genus, Hyphopichia genus, Kluyveromyces genus, Pichia genus, Schizoblastosporion genus, Schwanniomyces genus, Brevundimonas genus, Paenibacillus genus, Rhodotorula genus, Pseudomonas genus and Streptomyces genus. Further preferable examples are Candida albicans, Candida haemulonii, Candida intermedia, Candida maltosa, Candida mogii, Candida oleophila, Dipodascus ovetensis, Dipodascus tetrasperma, Geotrichum fragrans, Hypopichia burtonii, Kluyveromyces polysporus, Pichia stipitis, Schizoblastosporion kobayasii, Schwanniomyces occidentalis var. occidentalis, Brevundimonas diminuta, Paenibacillus alvei, Rhodotorula glutinis var. dairenensis, Pseudomonas stutzeri, Pseudomonas mendocina, Streptomyces coelescens and Streptomyces hydrogenans.

[0022] When obtaining 3-hydroxypentanenitrile whose absolute configuration is R-configuration, specific examples of the microorganism are Arthroascus javanensis IFO1848, Candida cantarellii IFO1261, Candida fennica CBS6087, Candida glabrata IFO0005, Candida gropengiesseri IFO0659, Candida kefyr IAM4880, Candida maris IFO10003, Candida melinii IFO0747, Candida musae IFO 1582, Candida pararugosa IFO0966, Candida pinus IFO0741, Candida

sorbophila IFO1583, Candida tenuis IFO0716, Candida utilis IFO0639, Cryptococcus curvatus IFO1159, Cryptococcus humicolus CBS2822, Debaryomyces hansenii IFO0063, Debaryomyces hansenii var. fabryi IFO0015, Debaryomyces hansenii var. hansenii IFO0032, Debaryomyces marama IFO0668, Debaryomyces nepalensis IFO0039, Dekkera anomala IFO0627, Geotrichum candidum CBS187-67, Geotrichum eriense ATCC22311, Geotrichum fermentans CBS452.83, Guilliermondella selenospora IFO1850, Issatchenkia orientalis IFO1279, Issatchenkia terricola IFO0933, Kluyveromyces marxianus IFO0288, Komagataella pastoris IFO0948, Komagataella pastoris IFO1013, Lipomyces starkeyi IFO0678, Lodderomyces elongisporus IFO1676, Metschnikowia bicuspidata IFO1408, Metschnikowia gruessii IFO0749, Ogataea pini IFO1342, Ogataea wickerhamii IFO1706, Pichia anomala IFO0120, Pichia anomala IFO0144, Pichia anomala IFO0146, Pichia canadensis IFO0976, Pichia jadinii IFO0987, Pichia petersonii IFO1372, Pichia rhodanensis IFO1272, Pichia silvicola IFO0807, Pichia triangularis IFO0836, Rhodotorula lactosa IFO1423, Rhodotorula rubra IFO0383, Rhodsporidium diobovatum IFO0688, Rhodsporidium sphaerocarpum IFO1438, Rhodsporidium toruloides IFO0413, Schwanniomyces occidentalis var. occidentalis IFO1840, Stephanoascus ciferrii IFO1854, Torulaspora delbrueckii IFO0381, Trichosporon cutaneum ATCC4151, Williopsis saturnus var. mrakii IFO0895, Williopsis saturnus var. saturnus IFO0992, Williopsis saturnus var. suaveolens IFO0809, Yarrowia lipolytica IFO1741, Acidephilium cryptum IFO14242, Agrobacterium tumefacience IFO12667, Agrobacterium tumefacience IFO13265, Alcaligenes sp. IFO14130, Achromobacter xylosoxidans subsp. denitrificans ATCC15173, Achromobacter xylosoxidans subsp. denitrificans IFO12669, Arthrobacter protophormiae IFO12128, Cellulomonas gelida IFO3748, Comamonas testosteroni IFO12048, Microbacterium arborescens IFO3750, Rhodococcus equi JCM1313, Rhodococcus erythropolis IAM1452, Rhodococcus erythropolis IFO12538, Rhodococcus erythropolis IFO12539, Rhodococcus rhodochrous IFO3338, Candida magnoliae IFO0705, Citeromyces matritensis IFO0651, Pichia bispora IFO0803, Trichosporon loubieri var. loubieri CBS7065, Corynebacterium ammoniagenes IFO12072, Corynebacterium flavescens IFO14136, Devosia riboflavina IFO13584, Hofnia alvei IFO3731, Proteus vulgaris IFO3167, Providencia alcalifaciens IFO12931, Absidia coerulea IFO4011, Absidia hyalospora IFO8082, Aegerita candida IFO6988, Agrocybe cylyndracea IFO30299, Amylostereum areolatum IFO9221, Aspergillus niger IFO4091, Aspergillus phoenicis IFO6670, Aspergillus sojae IFO4244, Corynascucs sepedonium IFO30067, Dendryphiella salina IFO8281, Emericella nidulans var. nidulans IFO4340, Emericella unguis IFO8087, Fusarium oxysporum IFO5942, Fusarium anguioides IFO4467, Gibberella fujikuroi IFO6603, Glomerella cingulata IFO5257, Macrophoma commelinae IFO9569, Micronectriella cucumeris IFO30005, Mortierella isabellina IFO7829, Mortierella ramanniana var. angulispora IFO6744, Mucor tuberculisporus IFO9256, Mucor inaequisporus IF08624, Nannizzia gypsea var. incurvata IFO8306, Penicillium chermesium IFO5800, Penicillium expansum IFO5854, Phialophora fastigiata IFO6850, Rhizopus niveus IFO4759, Rhizopus oryzae IF0O4705, Sclerotinia sclerotiorum IFO4876, Sclerotium delphinii IFO7337, Streptomyces cacaoi subsp. asoensis IFO13813 and Streptomyces sp. IFO13020. When obtaining 3-hydroxypentanenitrile whose absolute configuration is S-configuration, examples of the microorganism are Candida albicans IFO0759, Candida haemulonii IFO10001, Candida intermedia IFO0761, Candida maltosa IFO1977, Candida mogii IFO0436, Candida oleophila CBS2219, Dipodascus ovetensis IFO1201, Dipodascus tetrasperma CBS765.70, Geotrichum fragrans CBS 164.32, Hypopichia burtonii IFO0844, Kluyveromyces polysporus IF00996, Pichia stipitis CBS6054, Schizoblastosporion kobayasii IFO1644, Schwanniomyces occidentalis var. occidentalis IFO0371, Breyundimonas diminuta IFO12697, Paenibacillus alvei IFO3343, Rhodotorula glutinis var. dairenensis IFO0415, Pseudomonas stutzeri IFO13596, Pseudomonas mendocina IFO14162, Streptomyces coelescens IFO13378 and Streptomyces hydrogenans IFO13475.

[0023] These microorganisms can usually be obtained from easily obtainable stock strain and can also be isolated from nature. These microorganisms can also be mutated to obtain a strain having properties which are advantageous to the present reaction. Examples of properties which are advantageous to the present invention are improvement of specific activity to 3-ketopentanenitrile and improvement of stereoselectivity. Also, a gene which encodes an enzyme that asymmetrically reduces 3-ketopentanenitrile to optically active 3-hydroxypentanenitrile can be isolated from these microorganisms by a genetic engineering procedure and introduced into any microorganism.

[0024] For culturing these microorganisms, usually any medium containing a nutrition source that these microorganisms can assimilate can be used. For example, a common medium, in which a nutrition source, for example, carbon source such as saccharides including glucose, sucrose and maltose, organic acids including lactic acid, acetic acid, citric acid and propionic acid, alcohols including ethanol and glycerin, hydrocarbons including paraffin, fats including soya bean oil and rapeseed oil and mixtures thereof; nitrogen source such as ammonium sulfate, ammonium phosphate, urea, yeast extract, meat extract, peptone and corn-steep liquor; other inorganic salts and vitamins are mixed and compounded accordingly, can be used. The medium can be selected according to the type of microorganism which is used.

[0025] The microorganisms can generally be cultured under the usual conditions. For example, culturing aerobically in pH of 4.0 to 9.5 and a temperature range of 20 to 45°C for 10 to 96 hours is preferable. When the pH is less than 4.0 or more than 9.5 or the temperature is less than 20°C or more than 45°C, depending on the microorganism to be cultured, the microorganism may not proliferate or the proliferation rate may be extremely slow. When reacting a microorganism with 3-ketopentanenitrile, usually the culture solution itself containing cells of the microorganism can be

used for the reaction and concentrate of the culture solution can also be used. Examples of the concentration method are the method of collecting the cells from the culture solution by centrifugation or filtration and then suspending in a small amount of culture supernatant, water or buffer solution and the method of using a centrifugal concentrator. In the case that components in the culture solution affect the reaction, the cells obtained by centrifuging the culture solution or a treated substance thereof can be used.

[0026] The treated substance of the microorganism is not particularly limited and examples are dry cells obtained by dehydration with acetone or diphosphorus pentaoxide or by drying using a desiccator or fan, surfactant-treated substances, lytic enzyme-treated substances, immobilized cells or cell-free extract samples in which the cells are fractured. Furthermore, an enzyme that catalyzes the asymmetric reduction reaction can be purified from the culture and used.

[0027] In the reduction reaction, 3-ketopentanenitrile which is the substrate can be added all at once in the beginning of the reaction or divided into portions along with the progression of the reaction. As the substrate of the reaction, alkali metal salt of 3-ketopentanenitrile described below can be used as well. The temperature when reacting is preferably 10 to 60°C, more preferably 20 to 40°C and the pH when reacting is preferably 2.5 to 9, more preferably 5 to 9. When the temperature is less than 10°C or more than 60°C or the pH is less than 2.5 or more than 9, depending on the enzyme source which is used, the reaction may not progress or the reaction rate may become extremely slow.

[0028] The amount of the enzyme in the reaction solution can be determined according to the ability of the enzyme to reduce the substrate. The concentration of the substrate in the reaction solution is preferably 0.01 to 50 % (W/V), more preferably 0.1 to 30 % (W/V). When the concentration of the substrate is less than 0.01 % (W/V), the amount of 3-hydroxy-pentanetnitrile produced based on the reaction solution is small and efficiency is poor. When the concentration of the substrate is more than 50 % (W/V), there is a high possibility that unreacted substrates remain and productivity tends to become poor. The reaction is usually conducted by shaking or aeration agitation. The reaction time is determined according to the concentration of the substrate, the amount of the enzyme and other reaction conditions. Usually, each condition is preferably adjusted so that the reaction finishes in 2 to 168 hours.

[0029] In order to advance the reduction reaction, adding an energy source such as glucose, ethanol or isopropanol in a ratio of 0.5 to 30 % in the reaction solution is preferable, as excellent effects can be obtained. The reaction can also be advanced by adding a coenzyme such as reduced nicotinamide adenine dinucleotide (hereinafter referred to as NADH) and reduced nicotinamide adenine dinucleotide phosphate (hereinafter referred to as NADPH), which are usually considered to be necessary in a reduction reaction by a biological method. Specifically, in such a case, the coenzymes are added directly to the reaction solution.

[0030] Also, in order to advance the reduction reaction, reacting an enzyme, which reduces NAD^+ or $NADP^+$ to a reduced form, with a substrate for reducing by coexisting is preferable, as excellent results can be obtained. For example, glucose dehydrogenase as the enzyme that reduces to a reduced form and glucose as the substrate for reducing can coexist or formate dehydrogenase as the enzyme that reduces to a reduced form and formic acid as the substrate for reducing can coexist.

[0031] The amount of glucose used is to be at least equimolar to 3-ketopentanenitrile and the amount of glucose dehydrogenase is determined according to the relationship with activity of the reducing enzyme. In the same way, the amount of the formic acid is to be at least equimolar to 3-ketopentanenitrile and the amount of formate dehydrogenase is determined according to the relationship with activity of the reducing enzyme.

[0032] Also, adding a surfactant such as Triton (available from Nacalai Tesque, Inc.), Span (available from Kanto Kagaku) and Tween (available from Nacalai Tesque, Inc.) to the reaction solution is effective. Furthermore, in order to avoid inhibition of the reaction due to the substrate and/or alcohol body which is a product of the reduction reaction, a water-insoluble organic solvent such as ethyl acetate, butyl acetate, isopropyl ether, toluene and hexane can be added. In order to improve the solubility of the substrate, a water-soluble organic solvent such as methanol, ethanol, acetone, tetrahydrofurane and dimethylsulfoxide can be added.

[0033] The method for collecting optically active 3-hydroxypentanenitrile produced from the reduction reaction is not particularly limited. However, high-purity optically active 3-hydroxypentanenitrile can easily be obtained by directly extracting the reaction solution or extracting the substance obtained by separating cells from the reaction solution with a solvent such as ethyl acetate, toluene, t-butyl methyl ether or hexane, dehydrating and purifying by distillation or silica gel column chromatography.

[0034] After the conversion reaction, extraction is conducted with a suitable organic solvent and by analyzing the produced 3-hydroxypentanenitrile with capillary gas chromatography, the molar yield, absolute configuration and optical purity of the produced 3-hydroxypentanenitrile can be found.

[0035] Below, alkali metal salt of 3-ketopentanenitrile is described.

[0036] In the presence of an alkali metal base, 3-ketopentanenitrile is synthesized from propionic acid ester and acetonitrile. Preferable examples of the alkali metal base used are alkali metal base such as sodium ethoxide, sodium methoxide, sodium hydride, potassium ethoxide, potassium methoxide, potassium hydride and lithium hydride. Of these, in view of yield, sodium hydride is more preferable. Examples of the propionic acid ester are methyl propionate,

ethyl propionate and butyl propionate. Preferable examples of the catalyst when reacting are tetrahydrofurane, ether, benzene, ethanol and methanol are preferable. Of these, in view of yield, tetrahydrofurane is more preferable. The reaction temperature can be adjusted depending on the progression of the reaction and is preferably adjusted under reflux conditions.

[0037] The reaction is conducted under the above conditions and when production of 3-ketopentanenitrile is confirmed, alkali metal salt of 3-ketopentanenitrile can be precipitated as white crystal by cooling the reaction solution. Also, alkali metal salt of 3-ketopentanenitrile can be precipitated by adding a solvent that prevents alkali metal salt of 3-ketopentanenitrile from dissolving into the reaction solution. Examples of the solvent that prevents alkali metal salt of 3-ketopentanenitrile from dissolving are n-hexane, heptane and petroleum ether. The alkali metal salt of 3-ketopentanenitrile precipitated in the reaction solution can be isolated by filtering the reaction solution. The type of alkali metal of the obtained alkali metal salt of 3-ketopentanenitrile is the same as the type of alkali metal used in the synthesis reaction.

[0038] As described above, in addition to 3-ketopentanenitrile, the obtained alkali metal salt of 3-ketopentanenitrile can be used as a raw material when synthesizing optically active 3-hydroxypentanenitrile by action of an enzyme.

[0039] Hereinafter, the present invention is described in detail based on Examples but the present invention is not limited thereto. In the following descriptions, "%" represents "% by weight" unless specified otherwise.

EXAMPLE 1

[0040] A large scale test tube was charged with 5 ml of a liquid medium (pH 7) comprising 40 g of glucose, 3 g of yeast extract, 6.5 g of diammonium hydrogenphosphate, 1 g of potassium dihydrogenphosphate, 0.8 g of magnesium sulfate heptahydrate, 60 mg of zinc sulfate heptahydrate, 90 mg of iron sulfate heptahydrate, 5 mg of copper sulfate pentahydrate, 10 mg of manganese sulfate tetrahydrate and 100 mg of sodium chloride (all per 1 L) and sterilized by steam at 120°C for 20 minutes. One loop of the microorganisms shown in Table 1 were aseptically inoculated into the liquid solution and cultured by shaking at 30°C for 72 hours. After culturing, 2.5 ml of each culture solution was centrifuged to collect the cells of the microorganism and each of the cells were suspended in 0.5 ml of a 100 mM phosphate buffer solution (pH 6.5) containing 4 % of glucose. The cell suspension was added into a test tube in which 5 mg of 3-ketopentanenitrile was added in advance and reacted for 24 hours at 30°C. After the reaction, 1 ml of ethyl acetate was added to each reaction solution and mixed thoroughly and part of the organic layer was analyzed under the following capillary gas chromatography analysis conditions.

[Capillary gas chromatography analysis conditions]

[0041]

column: Chiraldex G-TA made by ASTEC, Inc. (20 m × 0.25 mm)
detection: FID
column temperature: 130°C
injection temperature: 200°C
detection temperature: 200°C
carrier gas: helium (100 kPa)
split ratio: 100/1
elution time: (R)-3-hydroxypentanenitrile 3.23 minutes, (S)-3-hydroxypentanenitrile 3.67 minutes

[0042] The molar yield, optical purity and absolute configuration of the produced 3-hydroxypentanenitrile are shown in Table 1.

TABLE 1

Microorganism			Molar yield (%)	Optical Purity (%e.e.)	Absolute Configuration
Arthroascus	javanensis	IFO 1848	5.9	85.1	R
Candida	cantarellii	IFO 1261	60.2	73.6	R
Candida	magnoliae	IFO 0705	30.0	77.0	R
Candida	glabrata	IFO 0005	5.3	82.4	R
Candida	gropengiesseri	IFO 0659	56.9	81.7	R
Candida	pararugosa	IFO 0966	39.1	83.1	R

TABLE 1 (continued)

Microorganism			Molar yield (%)	Optical Purity (%e.e.)	Absolute Configuration
Candida	pinus	IFO 0741	14.9	80.7	R
Candida	sorbophila	IFO 1583	41.0	74.7	R
Candida	fennica	CBS 6087	67.0	76.2	R
Candida	tenuis	IFO0716	9.0	75.0	R
Citeromyces	matritensis	IFO 0651	7.7	89.5	R
Cryptococcus	curvatus	IFO 1159	52.1	75.5	R
Cryptococcus	humicolus	CBS 2822	61.2	75.2	R
Debaryomyces	hansenii var. fabryi	IFO 0015	67.8	87.1	R
Debaryomyces	marama	IFO 0668	36.7	79.8	R
Debaryomyces	nepalensis	IFO 0039	44.1	94.8	R
Geotrichum	candidum	CBS 187.67	55.0	76.7	R
Geotrichum	erlense	ATCC 22311	60.1	74.6	R
Geotrichum	fermentans	CBS 452.83	58.2	78.7	R
Guilliermondella	selenospora	IFO 1850	63.7	77.3	R
Issatchenkia	terricola	IFO 0933	13.0	87.3	R
Komagataella	pastoris	IFO 0948	6.9	83.1	R
Komagataella	pastoris	IFO 1013	5.7	85.5	R
Lipomyces	starkeyi	IFO 0678	26.3	79.2	R
Ogataea	pini	IFO 1342	5.0	86.1	R
Pichia	anomala	IFO 0146	12.5	85.6	R
Pichia	silvicola	IFO 0807	68.0	75.7	R
Rhodsporidium	sphaerocarpum	IFO 1438	51.2	74.2	R
Rhodsporidium	toruloides	IFO 0413	72.1	76.9	R
Rhodotorula	rubra	IFO 0383	6.2	70.7	R
Trichosporon	cutaneum	ATCC 4151	18.4	94.0	R
Yarrowia	lipolytica	IFO 1741	6.2	82.3	R

EXAMPLE 2

[0043] The microorganisms shown in Table 2 were cultured and collected in the same manner as in Example 1. Cells of each microorganism were suspended in 0.5 ml of a 100 mM phosphate buffer solution (pH 6.5) containing 0.739 mg of NAD^+, 0.862 mg of $NADP^+$, 13.9 mg of glucose, 3 U of glucose dehydrogenase (product name: GLUCDH "Amano" II, available from Amano Enzyme, Inc.). The cell suspension was added into a test tube in which 5 mg of 3-ketopentanenitrile and 0.5 ml of butyl acetate were added in advance and reacted for 24 hours at 30°C. After the reaction, 0.5 ml of ethyl acetate was added to each reaction solution and mixed thoroughly and part of the organic layer was analyzed by the same analysis method as in Example 1. The molar yield, optical purity and absolute configuration of the produced 3-hydroxypentanenitrile are shown in Table 2.

TABLE 2

Microorganism			Molar yield (%)	Optical Purity (%e.e.)	Absolute Configuration
Candida	glabrata	IFO 0005	6.9	76.4	R
Candida	gropengiesseri	IFO 0659	11.3	83.0	R
Candida	kefyr	IAM 4880	19.1	76.9	R
Candida	pinus	IFO 0741	8.1	79.6	R
Candida	utilis	IFO 0639	18.2	81.0	R
Cryptococcus	humicola	CBS 2822	5.2	83.5	R
Debaryomyces	hansenii	IFO 0063	11.1	83.8	R

TABLE 2 (continued)

Microorganism			Molar yield (%)	Optical Purity (%e.e.)	Absolute Configuration
Debaryomyces	hansenii var. hansenii	IFO 0032	9.8	83.2	R
Debaryomyces	hansenii var. fabryi	IFO 0015	9.6	85.8	R
Dekkera	anomala	IFO 0627	9.7	86.0	R
Kluyveromyces	marxianus	IFO 0288	15.3	90.8	R
Komagataella	pastoris	IFO 0948	50.2	83.3	R
Metschnikowia	bicuspidata	IFO 1408	15.3	80.8	R
Metschnikowia	gruessii	IFO 0749	11.1	78.6	R
Pichia	anomala	IFO 0120	18.7	87.8	R
Pichia	anomala	IFO 0144	10.2	80.8	R
Pichia	bispora	IFO 0803	4.3	92.9	R
Pichia	jadinii	IFO 0987	29.9	78.1	R
Pichia	petersonii	IFO 1372	9.9	75.8	R
Pichia	silvicola	IFO 0807	24.1	76.2	R
Rhodotorula	lactosa	IFO 1423	6.5	76.3	R
Schwanniomyces	occidentalis var. occidentalis	IFO 1840	10.6	79.3	R
Stephanoascus	ciferrii	IFO 1854	8.7	76.7	R
Torulaspora	delbrueckii	IFO 0381	10.9	86.2	R
Trichosporon	loubieri var. loubieri	CBS 7065	7.7	85.1	R
Williopsis	saturnus var. suaveolens	IFO 0809	17.0	87.3	R
Williopsis	saturnus var. saturnus	IFO 0992	16.3	87.6	R
Yarrowia	lipolytica	IFO 1741	6.2	79.8	R
Candida	haemulonii	IFO 10001	18.3	82.8	S
Candida	albicans	IFO 0759	7.2	88.2	S
Dipodascus	ovetensis	IFO 1201	27.7	62.4	S
Dipodascus	tetrasperma	CBS 765.70	54.6	81.1	S
Geotrichum	fragrans	CBS 164.32	32.9	86.5	S
Hyphopichia	burtonii	IFO 0844	13.8	80.9	S
Kluyveromyces	polysporus	IFO 0996	3.3	74.7	S
Pichia	stipitis	CBS 6054	31.3	64.5	S
Rhodotorula	glutinis var. dairenensis	IFO 0415	5.7	75.7	S
Schwanniomyces	occidentalis var. occidentalis	IFO 0371	32.2	85.3	S

EXAMPLE 3

[0044] A 500 ml Sakaguchi flask was charged with 45 ml of a liquid medium comprising 40 g of glucose, 3 g of yeast extract, 6.5 g of diammonium hydrogenphosphate, 1 g of potassium dihydrogenphosphate, 0.8 g of magnesium sulfate heptahydrate, 60 mg of zinc sulfate heptahydrate, 90 mg of iron sulfate heptahydrate, 5 mg of copper sulfate pentahydrate, 10 mg of manganese sulfate tetrahydrate and 100 mg of sodium chloride (all per 900 ml) and 1 drop of adecanol and then sterilized. 5 ml of a sterilized 40 % glucose aqueous solution was added thereto and one loop of the microorganisms shown in Table 3 were aseptically inoculated and cultured by shaking at 30°C for 72 hours. After culturing, cells of the microorganisms were collected by centrifugation and washed twice with deionized water. The wet cells were suspended in 40 ml of deionized water. 1.2 L of acetone was added while stirring and cooling with ice and agitation

was conducted for 30 minutes in ice. The solution was filtered and the cells on the filter paper were washed with cooled acetone. Drying was conducted under reduced pressure and the acetone-dried cells of the microorganisms shown in Table 3 were respectively obtained.

[0045] With respect to each of the acetone-dried cells obtained by the above method, 10 mg of the acetone-dried cells, 0.739 mg of NAD^+, 13.9 mg of glucose, 3 U of glucose dehydrogenase (product name: GLUCDH "Amano" II, available from Amano Enzyme, Inc.), 0.5 ml of a 100 mM phosphate buffer solution (pH 6.5) and 5 mg of 3-ketopentanenitrile were added into a test tube and reacted for 24 hours at 30°C. After the reaction, 1 ml of ethyl acetate was added to each reaction solution and mixed thoroughly and part of the organic layer was analyzed by the same analysis method as in Example 1. The molar yield, optical purity and absolute configuration of the produced 3-hydroxypentanenitrile are shown in Table 3.

TABLE 3

Microorganism			Molar yield (%)	Optical Purity (%e.e.)	Absolute Configuration
Candida	maris	IFO 10003	22.0	79.0	R
Candida	melinii	IFO 0747	18.0	94.5	R
Issatchenkia	orientalis	IFO 1279	25.2	54.9	R
Issatchenkia	terricola	IFO 0933	8.2	73.7	R
Ogataea	pini	IFO 1342	3.8	73.4	R
Ogataea	wickerhamii	IFO 1706	13.9	71.7	R
Pichia	anomala	IFO 0120	31.1	88.7	R
Pichia	anomala	IFO 0144	14.0	79.0	R
Pichia	canadensis	IFO 0976	32.3	75.6	R
Williopsis	saturnus var. mrakii	IFO 0895	36.7	83.0	R
Williopsis	saturnus var. saturnus	IFO 0992	20.7	88.2	R
Williopsis	saturnus var. suaveolens	IFO 0809	54.0	83.8	R
Yarrowia	lipolytica	IFO 1741	12.8	78.7	R
Candida	intermedia	IFO 0761	23.2	75.7	S
Candida	maltosa	IFO 1977	31.8	91.5	S
Candida	mogii	IFO 0436	43.8	90.8	S
Candida	oleophila	CBS 2219	49.0	89.2	S
Candida	albicans	IFO 0759	23.5	93.5	S
Schizoblastosporion	kobayasii	IFO 1644	37.2	87.7	S

EXAMPLE 4

[0046] Acetone-dried cells of the microorganisms shown in Table 4 were respectively obtained in the same manner as in Example 3. With respect to each of the acetone-dried cells, 10 mg of the acetone-dried cells, 0.862 mg of $NADP^+$, 13.9 mg of glucose, 3 U of glucose dehydrogenase (product name: GLUCDH "Amano" II, available from Amano Enzyme, Inc.), 0.5 ml of a 100 mM phosphate buffer solution (pH 6.5) and 5 mg of 3-ketopentanenitrile were added into a test tube and reacted for 24 hours at 30°C. After the reaction, 1 ml of ethyl acetate was added to each reaction solution and mixed thoroughly and part of the organic layer was analyzed by the same analysis method as in Example 1. The molar yield, optical purity and absolute configuration of the produced 3-hydroxypentanenitrile are shown in Table 4.

TABLE 4

Microorganism			Molar yield (%)	Optical Purity (%e.e.)	Absolute Configuration
Candida	glabrata	IFO 0005	13.3	78.8	R
Candida	gropengiesseri	IFO 0659	30.1	79.7	R
Candida	melinii	IFO 0747	14.4	89.9	R

TABLE 4 (continued)

Microorganism			Molar yield (%)	Optical Purity (%e.e.)	Absolute Configuration
Candida	musae	IFO 1582	20.4	84.0	R
Candida	sorbophila	IFO 1583	21.8	79.5	R
Candida	tenuis	IFO 0716	11.1	81.4	R
Cryptococcus	humicola	CBS 2822	16.4	82.5	R
Debaryomyces	hansenii	IFO 0063	15.2	84.7	R
Debaryomyces	hansenii var. hansenii	IFO 0032	16.0	82.3	R
Debaryomyces	hansenii var. fabryi	IFO 0015	17.5	86.2	R
Dekkera	anomala	IFO 0627	14.3	80.5	R
Issatchenkia	orientalis	IFO 1279	17.4	50.8	R
Issatchenkia	terricola	IFO 0933	6.9	70.3	R
Lodderomyces	elongisporus	IFO 1676	12.5	77.4	R
Metschnikowia	bicuspidata	IFO 1408	15.3	79.3	R
Ogataea	pini	IFO 1342	4.5	76.8	R
Ogataea	wickerhamii	IFO 1706	6.8	59.7	R
Pichia	anomala	IFO 0120	32.2	88.2	R
Pichia	anomala	IFO 0144	10.3	76.5	R
Pichia	rbodanensis	IFO 1272	46.5	85.0	R
Pichia	triangularis	IFO 0836	11.8	79.9	R
Rhodsporidium	diobovatum	IFO 0688	21.4	81.1	R
Rhodsporidium	sphaerocarpum	IFO 1438	13.7	78.8	R
Rhodotorula	rubra	IFO 0383	28.0	76.3	R
Williopsis	saturnus var. saturnus	IFO 0992	14.8	87.1	R
Yarrowia	lipolytica	IFO 1741	36.9	81.4	R
Candida	mogii	IFO 0436	41.7	69.3	S
Dipodascus	tetrasperma	CBS 765.70	18.9	71.8	S

EXAMPLE 5

[0047] A large scale test tube was charged with 7 ml of a liquid medium (pH 7) comprising 10 g of meat extract, 10 g of peptone, 5 g of yeast extract and 3 g of sodium chloride (all per 1 L) and sterilized by steam at 120°C for 20 minutes. One loop of the microorganisms shown in Table 5 were aseptically inoculated into the liquid solution and cultured by shaking at 30°C for 36 hours. After culturing, 3.5 ml of each culture solution was centrifuged to collect cells of the microorganisms and each of the cells were suspended in 0.5 ml of a 100 mM phosphate buffer solution (pH 6.5) containing 8 % of glucose. The cell suspension was added into a test tube in which 5 mg of 3-ketopentanenitrile was added in advance and reacted for 18 hours at 30°C. After the reaction, analysis was conducted in the same manner as in Example 1. The molar yield, optical purity and absolute configuration of the produced 3-hydroxypentanenitrile are shown in Table 5.

TABLE 5

Microorganism			Molar yield (%)	Optical purity (%e.e.)	Absolute Configuration
Achromobacter	xylosoxidans subsp. denitrificans	IFO 12669	8.9	77.4	R
Achromobacter	xylosoxidans subsp. denitrificans	ATCC 15173	18.3	78.3	R

TABLE 5 (continued)

Microorganism			Molar yield (%)	Optical purity (%e.e.)	Absolute Configuration
Arthrobacter	protophormiae	IFO 12128	12.8	77.3	R
Acidiphilium	cryptum	IFO 14242	6.2	91.3	R
Cellulomonas	gelida	IFO 3748	7.1	84.1	R
Corynebacterium	ammoniagenes	IFO 12072	7.7	79.9	R
Corynebacterium	flavescens	IFO 14136	5.7	78.4	R
Devosia	riboflavina	IFO 13584	7.0	85.7	R
Microbacterium	arborescens	IFO 3750	6.3	71.0	R
Rhodococcus	erythropolis	IFO 12538	9.6	79.6	R
Rhodococcus	erythropolis	IFO 12539	5.0	70.3	R
Rhodococcus	erythropolis	IAM 1452	15.0	80.0	R
Rhodococcus	rhodochrous	IFO 3338	5.5	87.9	R

EXAMPLE 6

[0048] The microorganisms shown in Table 6 were cultured and collected in the same manner as in Example 5. Cells of each microorganism were suspended in 0.5 ml of a 100 mM phosphate buffer solution (pH 6.5) containing 0.739 mg of NAD^+, 0.862 mg of $NADP^+$, 13.9 mg of glucose and 3 U of glucose dehydrogenase (product name: GLUCDH "Amano" II, available from Amano Enzyme, Inc.). The cell suspension was added into a test tube in which 5 mg of 3-ketopentanenitrile and 0.5 ml of butyl acetate were added in advance and reacted for 24 hours at 30°C. After the reaction, 0.5 ml of ethyl acetate was added to each reaction solution and mixed thoroughly and part of the organic layer was analyzed by the same analysis method as in Example 1. The molar yield, optical purity and absolute configuration of the produced 3-hydroxypentanenitrile are shown in Table 6.

TABLE 6

Microorganism			Molar yield (%)	Optical Purity (%e.e.)	Absolute Configuration
Alcaligenes	sp.	IFO 14130	3.5	78.0	R
Agrobacterium	tumefacience	IFO 12667	3.6	73.4	R
Agrobacterium	tumefacience	IFO 13265	3.1	71.2	R
Comamonas	testosteroni	IFO 12048	14.3	78.5	R
Hafnia	alvei	IFO 3731	5.0	86.6	R
Proteus	vulgaris	IFO 3167	3.5	79.9	R
Providencia	alcalifaciens	IFO 12931	3.5	83.1	R
Rhodococcus	equi	JCM 1313	4.1	76.3	R
Brevundimonas	diminuta	IFO 12697	70.0	63.5	S
Paenibacillus	alvei	IFO 3343	5.7	76.4	S
Pseudomonas	stutzeri	IFO 13596	24	50.5	S
Pseudomonas	mendocina	IFO 14162	3.7	45.9	S

EXAMPLE 7

[0049] 22 g of 60 % sodium hydride was suspended in 400 ml of tetrahydrofurane. Then, while heating, 24.7 g of acetonitrile and subsequently 58.3 g of ethyl propionate were dropped and agitated overnight at 80°C. After naturally cooling to room temperature, the mixture was cooled further in ice water. The precipitated white crystal was obtained by filtration and dried under reduced pressure after washing with 350 ml of n-hexane. 45.0 g of white crystal 3-ketopentanenitrile·sodium salt was obtained.

EXAMPLE 8

[0050] 40 g of 60 % sodium hydride was suspended in 300 ml of tetrahydrofurane. Then, while heating, 49.3 g of

acetonitrile and subsequently 122.56 g of ethyl propionate were dropped and agitated overnight at 80°C. After naturally cooling to room temperature, 300 ml of n-hexane was added while cooling further in ice water. The precipitated white crystal was obtained by filtration and dried under reduced pressure after washing with 500 ml of n-hexane. 98.7 g of white crystal 3-ketopentanenitrile·sodium salt was obtained.

EXAMPLE 9

[0051] Candida gropengiesseri IFO0659 was cultured in the same manner as in Example 3 and 1 L of the obtained culture solution was centrifuged to collect cells of the microorganism. The cells were suspended in 200 ml of a 100 mM phosphate buffer solution (pH 6.5) containing 4 % of glucose. To this cell suspension, 1.19 g of 3-ketopentanenitrile·sodium salt was added while maintaining pH 6.5 using 6N hydrochloric acid. After adding, the reaction was conducted by agitating for 24 hours at 30°C. After the reaction, the aqueous phase was extracted with ethyl acetate and then extracted further with ethyl acetate. The organic phase was then combined and dehydration was conducted with anhydrous sodium sulfate. Thereafter, the solvent was removed under reduced pressure and purification was conducted by silica gel chromatography. 842 mg of 3-hydroxypentanenitrile was obtained. The optical purity found from the method described in Example 1 was 81.7 % e.e. in R-configuration. ^{1}H-NMR δ($CDCl_3$):1.00(3H,t), 1.64 (2H,dq), 2.27 (1H,s), 2.54 (2H,dd), 3.86-3.92 (1H,m).

EXAMPLE 10

[0052] The reaction and analysis were conducted in the same manner as in Example 1 except that the microorganisms shown in Table 7 were cultured in a medium (pH 6.0) comprising 5 % of glucose and 5 % of corn-steep liquor. The molar yield, optical purity and absolute configuration of the produced 3-hydroxypentanenitrile are shown in Table 7.

TABLE 7

Microorganism			Molar yield (%)	Optical purity (%e. e.)	Absolute configuration
Absidia	coerulea	IFO 4011	17.4	84.6	R
Absidia	hyalospora	IFO 8082	10.8	87.9	R
Aegerita	Candida	IFO 6988	6.1	86.8	R
Agrocybe	cylyndracea	IFO 30299	14.7	88.1	R
Amylostereum	areolatum	IFO 9221	12.9	87.8	R
Aspergillus	niger	IFO 4091	5.6	87.9	R
Aspergillus	phoenicis	IFO 6670	3.7	87.3	R
Aspergillus	sojae	IFO 4244	6.5	86.7	R
Corynascus	sepedonium	IFO 30067	17.2	80.0	R
Dendryphiella	salina	IFO 8281	12.8	81.6	R
Emericella	nidulans var. nidulans	IFO 4340	6.9	88.1	R
Emericella	unguis	IFO 8087	73.0	85.3	R
Fusarium	oxysporum	IFO 5942	35.7	88.5	R
Fusarium	anguioides	IFO 4467	13.2	84.9	R
Gibberella	fujikuroi	IFO 6603	16.1	85.7	R
Glomerella	cingulata	IFO 5257	21.7	86.1	R
Macrophoma	commelinae	IFO 9569	40.6	74.1	R
Micronectriella	cucumeris	IFO 30005	26.7	80.8	R
Mortierella	isabellina	IFO 7829	59.9	85.2	R
Mortierella	ramanniana var. angulispora	IFO 6744	23.1	86.2	R
Mucor	tuberculisporus	IFO 9256	61.0	82.6	R
Mucor	inaequisporus	IFO 8624	56.5	84.0	R
Nannizzia	gypsea var. incurvata	IFO 8306	20.5	87.6	R
Penicillium	chermesium	IFO 5800	26.7	86.9	R

TABLE 7 (continued)

Microorganism			Molar yield (%)	Optical purity (%e.e.)	Absolute configuration
Penicillium	expansum	IFO 5854	14.9	85.8	R
Phialophora	fastigiata	IFO 6850	8.6	87.4	R
Rhizopus	niveus	IFO 4759	12.9	82.0	R
Rhizopus	oryzae	IFO 4705	13.0	81.8	R
Sclerotinia	sclerotiorum	IFO 4876	6.0	87.7	R
Sclerotium	delphinii	IFO 7337	13.9	87.2	R

EXAMPLE 11

[0053] The reaction and analysis were conducted in the same manner as in Example 1 except that the microorganisms shown in Table 8 were cultured in a medium (pH 7.2) comprising 3 % of Tryptic Soy Broth available from Difco Laboratories and 1 % of soluble starch. The molar yield, optical purity and absolute configuration of the produced 3-hydroxypentanenitrile are shown in Table 8.

TABLE 8

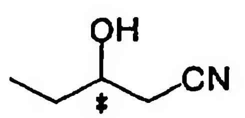
Microorganism			Molar yield (%)	Optical purity (%e.e.)	Absolute configuration
Streptomyces	cacaoi subsp. asoensis	IFO 13813	5.3	64.5	R
Strentomyces	sp.	IFO 13020	3.7	53.7	R
Streptomyces	coelescens	IFO 13378	12.2	39.2	S
Streptomyces	hydrogenans	IFO 13475	3.5	51.5	S

INDUSTRIAL APPLICABILITY

[0054] According to the present invention, optically active 3-hydroxypentanenitrile can be prepared with high yield by stereoselectively reducing 3-ketopentanenitrile by action of an enzyme having asymmetric reduction activity. Also, alkali metal salt of 3-ketopentanenitrile, which is a stable compound without problems regarding storage, can be efficiently obtained.

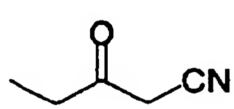
Claims

1. A process for preparing optically active 3-hydroxypentanenitrile represented by the following formula (1):



(1)

wherein an enzyme, which asymmetrically reduces 3-ketopentanenitrile to optically active 3-hydroxypentanenitrile, acts upon 3-ketopentanenitrile represented by the following formula (2):



(2)

to obtain optically active 3-hydroxypentanenitrile.

2. The process of Claim 1, wherein said enzyme is an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Arthroascus genus, Candida genus, Cryptococcus genus, Debaryomyces genus, Dekkera genus, Dipodascus genus, Geotrichum genus, Guilliermondella genus, Hyphopichia genus, Issatchenkia genus, Kluyveromyces genus, Komagataella genus, Lipomyces genus, Lodderomyces genus, Metschnikowia genus, Ogataea genus, Pichia genus, Rhodotorula genus, Rhodsporidium genus, Schizoblastosporion genus, Schwanniomyces genus, Stephanoascus genus, Torulaspora genus, Trichosporon genus, Williopsis genus, Yarrowia genus, Acidephilium genus, Agrobacterium genus, Alcaligenes genus, Arthrobacter genus, Brevundimonas genus, Cellulomonas genus, Comamonas genus, Microbacterium genus, Paenibacillus genus, Rhodococcus genus, Citeromyces genus, Achromobacter genus, Corynebacterium genus, Devosia genus, Hofnia genus, Proteus genus, Providencia genus, Pseudomonas genus, Absidia genus, Aegerita genus, Agrocybe genus, Amylostereum genus, Aspergillus genus, Corynascucs genus, Dendryphiella genus, Emericella genus, Fusarium genus, Gibberella genus, Glomerella genus, Macrophoma genus, Micronectriella genus, Mortierella genus, Mucor genus, Nannizzia genus, Penicillium genus, Phialophora genus, Rhizopus genus, Sclerotinia genus, Sclerotium genus and Streptomyces genus; and/or a purified enzyme obtained from said microorganism.

3. The process of Claim 1, wherein absolute configuration of said produced optically active 3-hydroxypentanenitrile is R-configuration and said enzyme is an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Arthroascus genus, Candida genus, Cryptococcus genus, Debaryomyces genus, Dekkera genus, Geotrichum genus, Guilliermondella genus, Issatchenkia genus, Kluyveromyces genus, Komagataella genus, Lipomyces genus, Lodderomyces genus, Metschnikowia genus, Ogataea genus, Pichia genus, Rhodotorula genus, Rhodsporidium genus, Schwanniomyces genus, Stephanoascus genus, Torulaspora genus, Trichosporon genus, Williopsis genus, Yarrowia genus, Acidephilium genus, Agrobacterium genus, Alcaligenes genus, Arthrobacter genus, Cellulomonas genus, Comamonas genus, Microbacterium genus, Rhodococcus genus, Citeromyces genus, Achromobacter genus, Corynebacterium genus, Devosia genus, Hofnia genus, Proteus genus, Providencia genus, Absidia genus, Aegerita genus, Agrocybe genus, Amylostereum genus, Aspergillus genus, Corynascucs genus, Dendryphiella genus, Emericella genus, Fusarium genus, Gibberella genus, Glomerella genus, Macrophoma genus, Micronectriella genus, Mortierella genus, Mucor genus, Nannizzia genus, Penicillium genus, Phialophora genus, Rhizopus genus, Sclerotinia genus, Sclerotium genus and Streptomyces genus; and/or a purified enzyme obtained from said microorganism.

4. The process of Claim 1, wherein absolute configuration of said produced optically active 3-hydroxypentanenitrile is R-configuration and said enzyme is an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Arthroascus javanensis, Candida cantarellii, Candida fennica, Candida glabrata, Candida gropengiesseri, Candida kefyr, Candida maris, Candida melinii, Candida musae, Candida pararugosa, Candida pinus, Candida sorbophila, Candida tenuis, Candida utilis, Cryptococcus curvatus, Cryptococcus humicolus, Debaryomyces hansenii, Debaryomyces hansenii var. fabryi, Debaryomyces hansenii var. hansenii, Debaryomyces marama, Debaryomyces nepalensis, Dekkera anomala, Geotrichum candidum, Geotrichum eriense, Geotrichum fermentans, Guilliermondella selenospora, Issatchenkia orientalis, Issatchenkia terricola, Kluyveromyces marxianus, Komagataella pastoris, Lipomyces starkeyi, Lodderomyces elongisporus, Metschnikowia bicuspidata, Metschnikowia gruessii, Ogataea pini, Ogataea wickerhamii, Pichia anomala, Pichia canadensis, Pichia jadinii, Pichia petersonii, Pichia rhodanensis, Pichia silvicola, Pichia triangularis, Rhodotorula lactosa, Rhodotorula rubra, Rhodsporidium diobovatum, Rhodsporidium sphaerocarpum, Rhodsporidium toruloides, Schwanniomyces occidentalis var. occidentalis, Stephanoascus ciferrii, Torulaspora delbrueckii, Trichosporon cutaneum, Williopsis saturnus var. mrakii, Williopsis saturnus var. saturnus, Williopsis saturnus var. suaveolens, Yarrowia lipolytica, Acidephilium cryptum, Agrobacterium tumefacience, Alcaligenes sp., Achromobacter xylosoxidans subsp. denitrificans, Arthrobacter protophormiae, Cellulomonas gelida, Comamonas testosteroni, Microbacterium arborescens, Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus rhodochrous, Candida magnoliae, Citeromyces matritensis, Pichia bispora, Trichosporon loubieri var. loubieri, Corynebacterium ammoniagenes, Corynebacterium flavescens, Devosia riboflavina, Hofnia alvei, Proteus vulgaris, Providencia alcalifaciens, Absidia coerulea, Absidia hyalospora, Aegerita Candida, Agrocybe cylyndracea, Amylostereum areolatum, Aspergillus niger, Aspergillus phoenicis, Aspergillus sojae, Corynascucs sepedonium, Dendryphiella salina, Emericella nidulans var. nidulans, Emericella unguis, Fusarium oxysporum, Fusarium anguioides, Gibberella fujikuroi, Glomerella cingulata, Macrophoma commelinae, Micronectriella cucumeris, Mortierella isabellina, Mortierella ramanniana var. angulispora, Mucor tuberculisporus, Mucor inaequisporus, Nannizzia gypsea var. incurvata, Penicillium chermesium, Penicillium expansum, Phialophora fastigiata, Rhizopus niveus, Rhizopus oryzae,

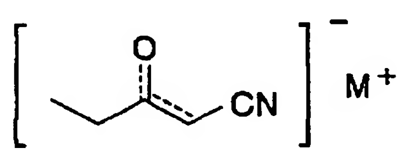
Sclerotinia sclerotiorum, Sclerotium delphinii, Streptomyces cacaoi subsp. asoensis and Streptomyces sp.; and/or a purified enzyme obtained from said microorganism.

5. The process of Claim 1, wherein absolute configuration of said produced optically active 3-hydroxypentanenitrile is S-configuration and said enzyme is an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Candida genus, Dipodascus genus, Geotrichum genus, Hyphopichia genus, Kluyveromyces genus, Pichia genus, Schizoblastosporion genus, Schwanniomyces genus, Brevundimonas genus, Paenibacillus genus, Rhodotorula genus, Pseudomonas genus and Streptomyces genus; and/or a purified enzyme obtained from said microorganism.

6. The process of Claim 1, wherein absolute configuration of said produced optically active 3-hydroxypentanenitrile is S-configuration and said enzyme is an enzyme present in a cell, a culture solution or a treated substance thereof of a microorganism selected from the group consisting of Candida albicans, Candida haemulonii, Candida intermedia, Candida maltosa, Candida mogii, Candida oleophila, Dipodascus ovetensis, Dipodascus tetrasperma, Geotrichum fragrans, Hypopichia burtonii, Kluyveromyces polysporus, Pichia stipitis, Schizoblastosporion kobayasii, Schwanniomyces occidentalis var. occidentalis, Brevundimonas diminuta, Paenibacillus alvei, Rhodotorula glutinis var. dairenensis, Pseudomonas stutzeri, Pseudomonas mendocina, Streptomyces coelescens and Streptomyces hydrogenans; and/or a purified enzyme obtained from said microorganism.

7. The process of Claim 1, 2, 3, 4, 5 or 6, wherein either or both of oxidized nicotinamide adenine dinucleotide (NAD^+) and oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) coexist with an enzyme that reduces each to a reduced form and a substrate for reducing.

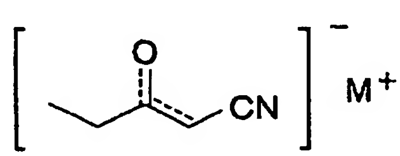
8. The process of Claim 1, 2, 3, 4, 5, 6 or 7, wherein, an alkali metal salt of 3-ketopentanenitrile represented by the following formula (3):



(3)

(wherein M represents an alkali metal) is used as 3-ketopentanenitrile.

9. A process for preparing an alkali metal salt of 3-ketopentanenitrile, which comprises synthesizing 3-ketopentanenitrile from propionic acid ester and acetonitrile in the presence of an alkali metal base and obtaining 3-ketopentanenitrile from the reaction system as an alkali metal salt represented by the following formula (3):



(3)

(wherein M represents an alkali metal).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/10312

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl⁷ C12P13/00//(C12P13/00, C12R1:01)(C12P13/00, C12R1:025)(C12P13/00, C12R1:05)(C12P13/00,C12R1:06)(C12P13/00, C12R1:15)(C12P13/00, C12R1:32)(C12P13/00, C12R1:37)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl⁷ C12P13/00-17/18, C07C255/11-255/14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
BIOSIS/WPI(DIALOG), CA/REGISTRY(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94/21617 A1 (DOWELANCO), 29 September, 1994 (29.09.94), & AU 9465186 A	1-8
A	ITOH T. et al., Thiacrown Ether Technology in Lipase-Catalyzed Reaction: Scope and Limitation for Preparing Optically Active 3-Hydroxyalkanenitriles and Application to Insect Pheromone Synthesis. J. Org. Chem. Vol.62, No.26 (1997) pages 9165 to 9172	1-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 December, 2002 (27.12.02)	28 January, 2003 (28.01.03)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/10312

Continuation of A. CLASSIFICATION OF SUBJECT MATTER
(International Patent Classification (IPC))

Int.Cl^7 (C12P13/00, C12R1:38)(C12P13/00, C12R1:465)(C12P13/00, C12R1:645)(C12P13/00, C12R1:65)(C12P13/00, C12R1:66)(C12P13/00, C12R1:72)(C12P13/00, C12R1:77)(C12P13/00, C12R1:785)(C12P13/00, C12R1:80)(C12P13/00, C12R1:84)(C12P13/00, C12R1:845)

(According to International Patent Classification (IPC) or to both national classification and IPC)

Form PCT/ISA/210 (extra sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/10312

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Claims 1-9 include two inventions, i.e., an invention related to a process for producing optically active 3-hydroxypentanenitrile by making an enzyme capable of catalyzing asymmetric reduction act on 3-ketopentanenitrile as set forth in claims 1-8, and an invention related to a process for producing stable 3-ketopentanenitrile alkali metal salts as set forth in claim 9, and 3-ketopentanenitrile which is the main feature common to the two inventions is a publicly known compound. Further, both the inventions are not considered as having a special technical feature in common. Thus, the inventions are not considered as being so linked as to form a single general inventive concept.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-8

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)